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Convergent evolution underground - two new troglomorphic collembolan species of the genus *Schaefferia* Absolon, 1900 (Collembola, Poduromorpha, Hypogastruridae) from Iran and Montenegro

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Abstract

Two new subterranean springtail species of the genus *Schaefferia* Absolon, 1900 are described: *S. greensladeae* **sp. nov.** from Danial Cave in the Alborz Mountains, Iran, and *S. montenegrina* **sp. nov.** from Babatuša Cave in Dinaric Karst, Montenegro. Both species are characterized by the absence of eyes and postantennal organ (PAO) having more than 5 lobes in the contrary to other eyeless congeners that have less than 5 lobes in PAO. Morphological traits of both species indicate high level of association with the subterranean environment. The phylogenetic relationships and morphological adaptations of two new species to subterranean life are discussed. *Schaefferia greensladeae* **sp. nov.** and *S. montenegrina* **sp. nov.** serve as a good example of convergent evolution that is characteristic of invertebrates highly adapted to deep subterranean realm. An updated key to the world species of the genus and a table summarizing diagnostic characters of the world species of *Schaefferia* are provided.

Keywords

Cave springtails, convergent evolution, phylogeny, *Schaefferia*, taxonomy

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Introduction

The genus *Schaefferia* Absolon, 1900 (Hypogastruridae) was first established for *S. emucronata*, a species described from a cave in the Czech Republic. The genus is characterized by the following features: body pigment reduced, eyes from 0+0 to 7+7 (mostly less than 5+5), furca reduced or absent - if present, then dens usually with 3 or 4 setae (sometimes 5 or 6), anal spines long (more or less equal to claw in length), Ant IV with a simple apical bulb and 5 to 12 sensilla, body covered with well-differentiated setae, Ti I–III without knobbed tenent hairs, claws with or without inner tooth, empodium well-developed with basal lamella. Moreover, the genus belongs to the *Ceratophysella denticulata* line, i.e. its representatives are characterized by a specific setal pattern (Thibaud et al. 2004).

Schaefferia emucronata Absolon, 1900 is the nominal species of the genus, described from Kateřinská jeskyně Cave in the Moravian Karst, Czech Republic. Later, *S. willemi* (Bonet, 1930) was described from the Rochefort and Han caves in Belgium and two species from caves in Germany: *S. decemoculata* (Stach, 1939) from Kellerhöhle, Westfalia and *S. quadrioculata* (Stach, 1939) from Diebshöhle and Heimkehle caves in Harz Mountains. The first species that was found to inhabit surface environment was *S. pouadensis* Delamare Deboutteville, 1945 from France. *Schaefferia guerrerense* (Bonet, 1945) was the first species reported outside Europe, discovered in Mexico in the popular Cacahuamilpa show cave.

The genus has Holarctic distribution (Thibaud 1972) with 35 species described up to date (Jordana et al. 2020), involving both edaphic and subterranean species. The representatives of the genus often inhabit caves in low altitudes, while humus, soil and mosses in mountains, being frequent in relatively cold microclimate with air temperature around 10 °C and relatively high humidity (Thibaud 1972). A recent study, carried out by Jordana et al. (2020) in Sierra de Gaudarrama Mountains, Spain, yielded a species associated with colluvial "milieu souterrain superficiel" (MSS - mesovoid shallow substratum) documenting variety of subterranean habitats that the congeners of this genus occupy.

In this paper, we provide comprehensive descriptions of two new eyeless *Schaefferia* species that were discovered during the recent cave explorations in Iran and Montenegro, with their morphological traits showing high level of association with the subterranean environment. A table summarizing diagnostic characters of eyeless *Schaefferia* congeners is included together with an updated key to the world species of the genus. The phylogenetic relationships and morphological adaptations of two new species to subterranean life are discussed.

Material and methods

Cave descriptions

Danial Cave (IRAN)

Danial Cave is the second largest river cave in Iran, which is located in the Alborz Mountain range in the Mazandaran karst area, on the southern edge of the Caspian Sea near the Salman Shahr of Abbasabad city (36°39'35.2"N, 51°10'53.2"E) at altitude of 204 m above sea level (Fig. 1), its name derived from the close village. Alternative name of this cave is Gerde Kooh. The cave is situated in the Hyrcanian Forest type on the slope of the mountain with the main entrance 5 m long and 6 m high (Fig. 2). Its length is 2,158 m, with a subterranean river flowing along the entire cave (Yusefinia 2017; Ghelich Khani et al. 2022; Barjadze et al. 2024; Mehrafrooz Mayvan et al. 2025). It has constant air temperature (around 12 °C) and relative humidity (RH) is over 73%, measured with a thermo-hygrometer TFA 30.5015 DTH in April 2024.

Babatuša Cave (MONTENEGRO)

This cave is situated near the village of Trnovo, Virpazar, (42°17'56"N, 19°01'58"E) at altitude of 381 m above sea level (Fig. 7). The entrance is a horizontal fissure about 10 m wide and about 2 m high (Fig. 8) (Vujačić et al. 2024). The cave is relatively short (a little over 290 m), with the great main chamber rich in flowstones and small sinter pools (Djurovic and Djurovic 2021). Its galleries are filled with clay deposits up to 10 m thick (Vujačić et al. 2024).

Methods

For observation in an optical microscope with phase contrast, specimens were firstly boiled softly in evaporation glass dish with 95% ethyl alcohol on electric cooking plate for approximately 1 minute to remove fat from the body. For clearing, the specimens were transported to concave glass dish with 10% water solution of KOH for ca. 1 minute and then transported to a dish containing Chlorophenol until the specimens were fully transparent. At the end, specimens were mounted on Swann medium on permanent slides. After slide was sufficiently dried out, the cover glass was ringed with Canada balsam to seal the mounting medium and prevent its desiccation. The specimens were observed in Leica DM 2500 light microscope equipped with phase and DIC contrasts, and a drawing arm.

Abbreviations used. ***a***—anterior row of seta(e); **Ant**—antennal segment(s); **Asp**—anal spines; **Cl**—claw; ***m***—median row of seta(e); **Mc**—macroseta(e); **ms**—microsensillum; **os**—subapical organite (sub-apical sensory peg); ***p***—posterior row of seta(e); **PAO**—postantennal organ; **s**—sensory seta; **Ti**—tibiotarsus; **VT**—ventral tube; **CoPJSU**—Collembola collection of the Department of Zoology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University, Košice, Slovakia; **ZMFUM**—Zoology Museum of Ferdowsi University of Mashhad, Mashhad, Iran.

Results

Species description

Schaefferia greensladeae **Mehrafrooz Mayvan & Kováč, sp. nov.**
https://zoobank.org/424A45B7-3B5F-41C2-992B-788B3B16DC29
Figs 3–6, Table 1

Diagnosis. Eyes absent, PAO large with 7 subequal lobes formed around central circle. Ant IV with 7–8 sensilla and simple apical vesicle. Claw enlarged, with an internal tooth and pair of lateral teeth. Retinaculum with 3–4 teeth, furca well-developed, with 5 dental setae.

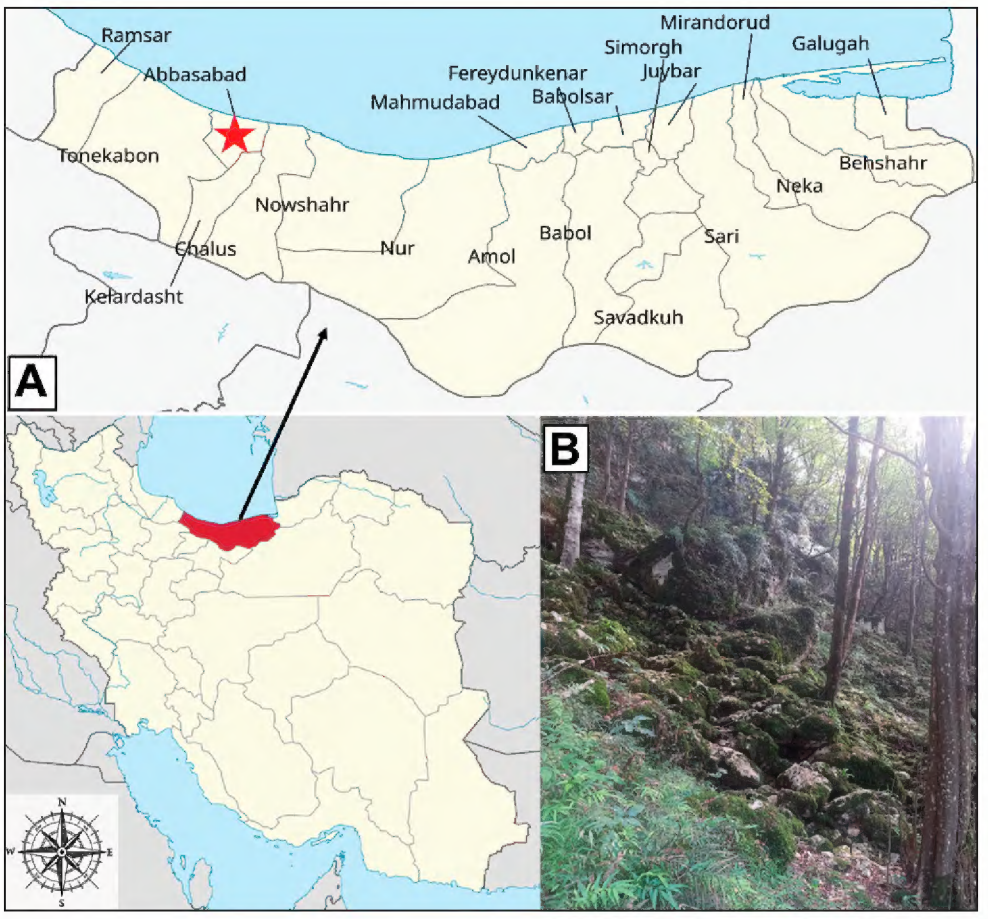

Figure 1. A map of Iran, red area - Mazandaran province; location of the Danial Cave, *locus typicus* of *Schaefferia greensladeae* sp. nov., is marked with a red asterisk on an enlarged map of the province (upper) **B** Hyrcanian Forest near the cave in summer (Photo: M. Mehrafrooz).

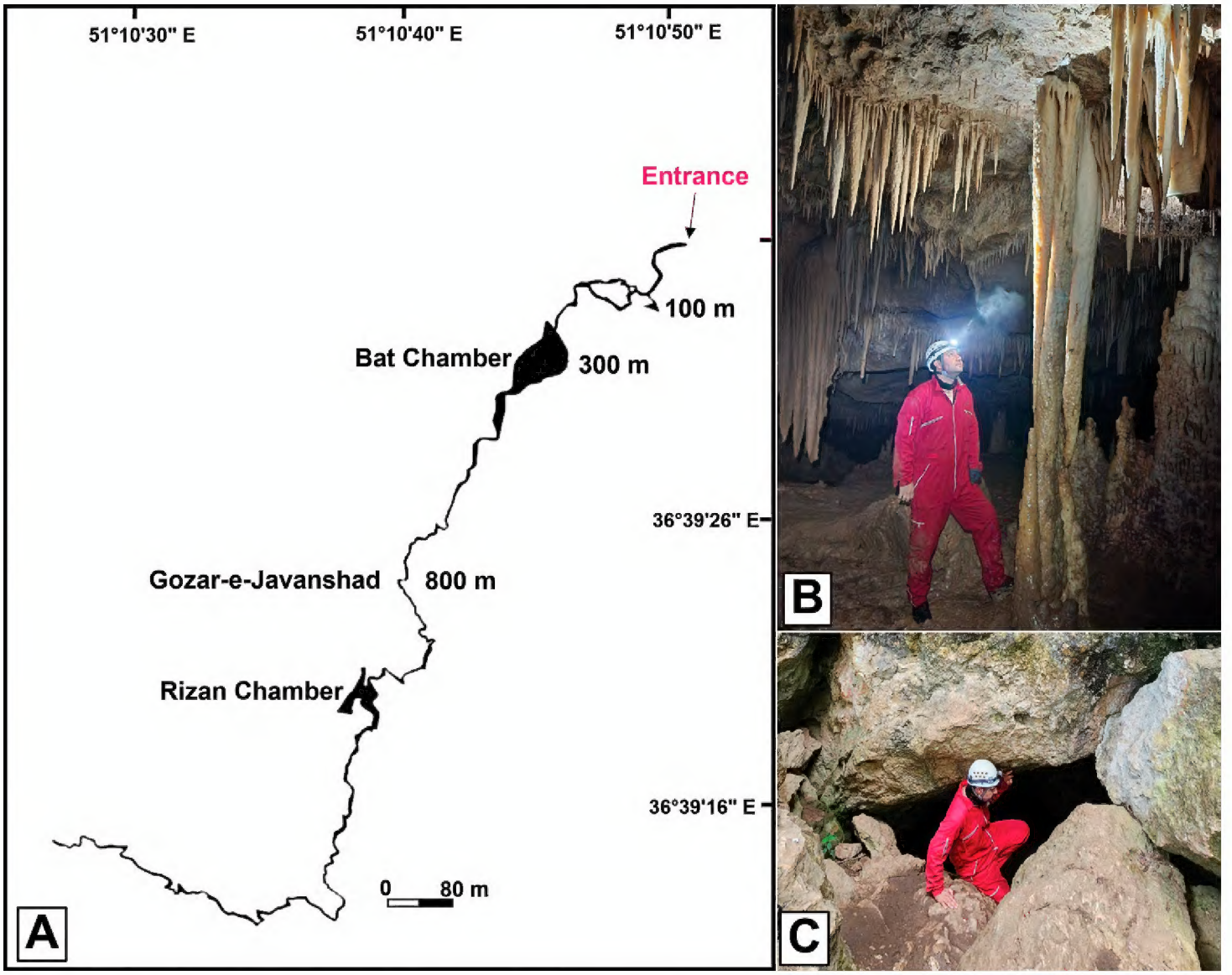

Figure 2. A ground plan of the Danial Cave (adopted from Ghelich Khani et al. 2024) **B** cave decorations in Rizan chamber (Photo: M. Mehrafrooz) **C** main entrance of the cave (Photo: M. Mehrafrooz).

Type locality. Iran, Mazandaran province, Salman Shahr County, Abbasabad city, Danial village, Danial Cave, Coordinates: 36°39'35.2"N, 51°10'53.2"E, cave entrance 204 m a.s.l.

Type material. ***Holotype*:** • Female on slide [DaSc101IR], dark zone, collected from surface of water puddle, 15.x.2023, temperature = 11.9 °C, RH 73.0%, leg. M. Mehrafrooz. ***Paratypes*:** • 2 specimens on slides [DaSc102IR and DaSc103IR], dark zone, collected from surface of water puddle, 15.x.2023, temperature = 12.0 °C, RH 73.4%, leg. M. Mehrafrooz. Holotype deposited in CoPJSU; two paratypes deposited in ZMFUM.

Description. ***Body appearance.*** Length 1.9 mm on average (n = 3, max. length 2 mm). Habitus typical of *Ceratophysella* Börner in Brohmer, 1932 without eyes. Colour alive white without traces of pigment. Cuticular granulation rather uniform, slightly coarser on Abd V–VI, macro- and mesosetae differentiated.

Antenna. It is nearly as long as head, with apical bulb (Fig. 3A–D). Length of Ant I, II, III, IV in holotype as 0.07, 0.06, 0.07, 0.1 mm, respectively. Ant I with 7 setae; Ant II with 6 dorsal and 6 ventral setae in a single row and 1 short smooth seta near its base (dorsal setae clearly coarser and thicker); Ant III and IV clearly

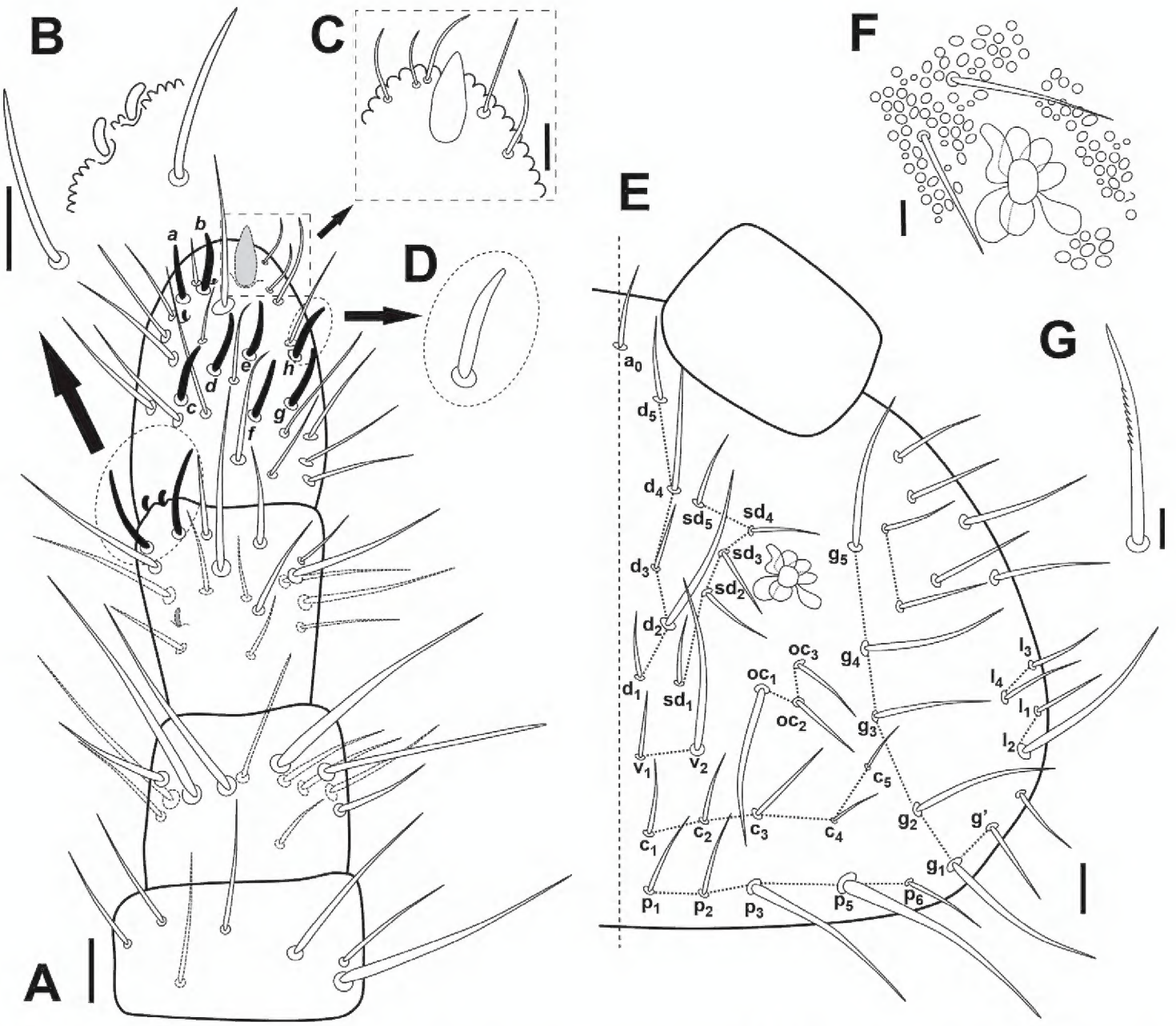

Figure 3. *Schaefferia greensladeae* sp. nov. **A** antennal segments I–IV **B** Ant III sensory organ **C** tip of antenna **D** Ant IV blunt sensillum **E** dorsal side of head, setal pattern **F** postantennal organ with the nearest setae and granulation of cuticle **G** shape of macroseta. Scale bars: 0.025 mm (**A**); 0.01 mm (**B, C**); 0.025 mm (**E**); 0.02 mm (**F–G**).

separated, Ant III sensory organ with 2 central short and curved rods flanked by 2 long guard sensilla (Fig. 3B), a small ventrolateral microsensillum in a ventral pit, and 7 dorsal and 8 ventral ordinary setae; Ant IV with a simple apical bulb (Fig. 3C), 7–8 cylindrical blunt sensilla (*a*, *b*, *c*, *d*, *e*, *f*, *g* and *h*) (after Yosii 1962), subapical ms near base of sensillum *a*, apical organite os placed near sensillum *b* and 20 setae around sensilla.

Head. Eyes absent. PAO near base of Ant I well developed, large, about 33 μm in diameter, with 7 subequal lobes formed around central circle (Fig. 3F). Labrum typical of the genus with 2+2 prelabral and 5, 5, 4 labral setae. Labium with all normal papillae (A–E) and 6 proximal setae. Left mandible with 4 teeth and right mandible with 5 teeth.

Abdomen. Ventral tube with 4+4 apical setae. Retinaculum with 3–4 teeth (asymmetry observed). Furca well-developed (Fig. 5B), length of manubrium, dens,

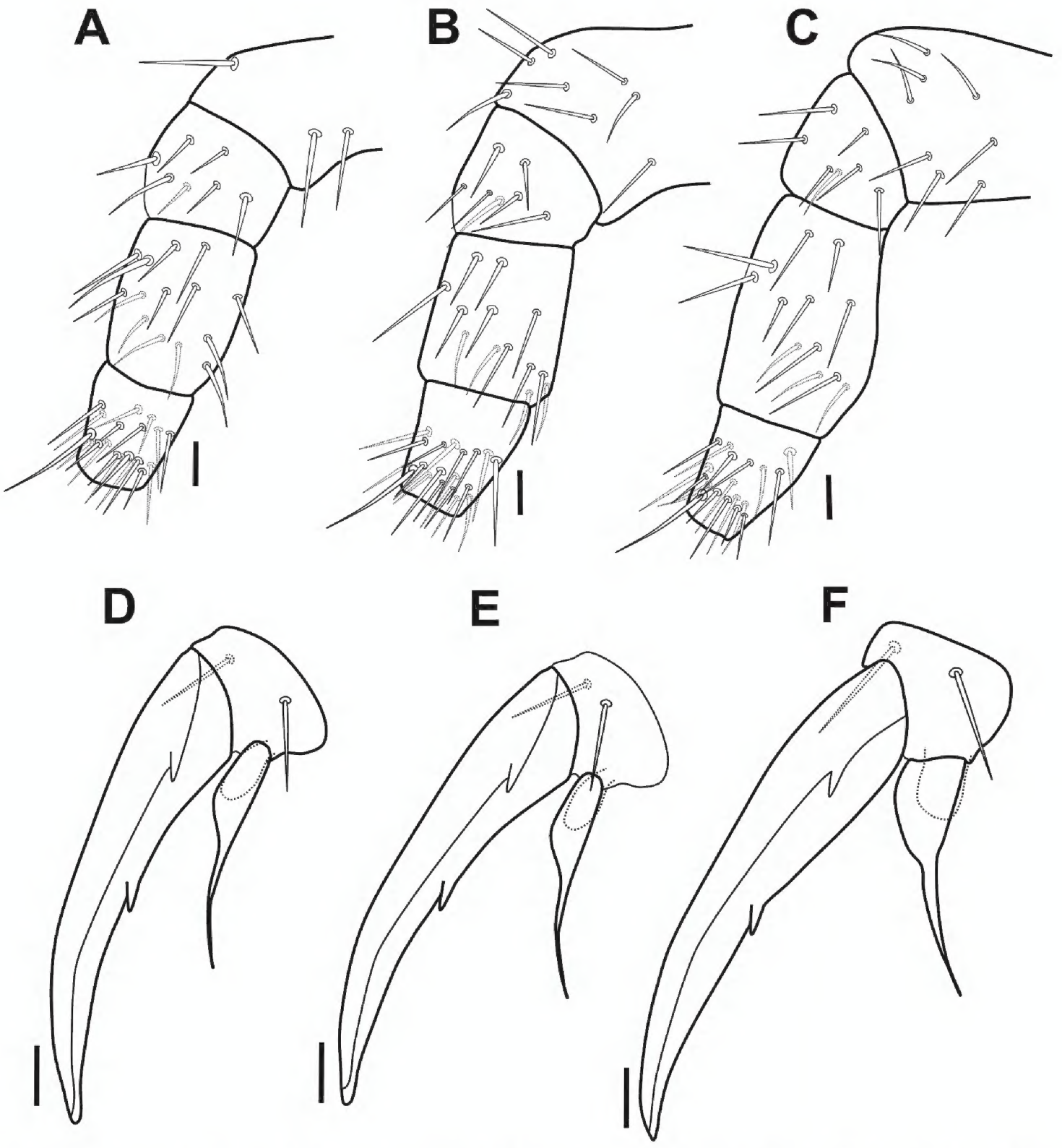

Figure 4. *Schaefferia greensladeae* sp. nov. **A–C** setal pattern of legs **A** leg I **B** leg II **C** leg III **D–F** foot complex of legs **D** leg I **E** leg II **F** leg III. Scale bars: 0.025 mm (**A–C**); 0.01 mm (**D–F**).

mucro in holotype as 0.11, 0.06, 0.01 mm, respectively; manubrium with 12 setae; dens with 5 setae (basal seta longer); mucro about 1/4 of dens length, with lateral lamella, mucro and dens taken together are as long as inner edge of claw III. Anal spines long (86 μm), on papillae (Fig. 6C). Ratio anal spines (including papilla): claw III = 1.6; ratio anal spines: dens = 1.9.

Legs. Legs I–III with normal setae, tenent hair acuminate. Setal pattern as in Fig. 4A–C. Leg I–III with 3, 8, 8 setae on coxae, 7, 7, 7 setae on trochanters, 14, 13, 12 setae on femora and 19, 19, 18 setae on tibiotarsi, respectively. Claw slender, 78 μm long in holotype, internal edge with strong medial tooth and lateral edge with pair of

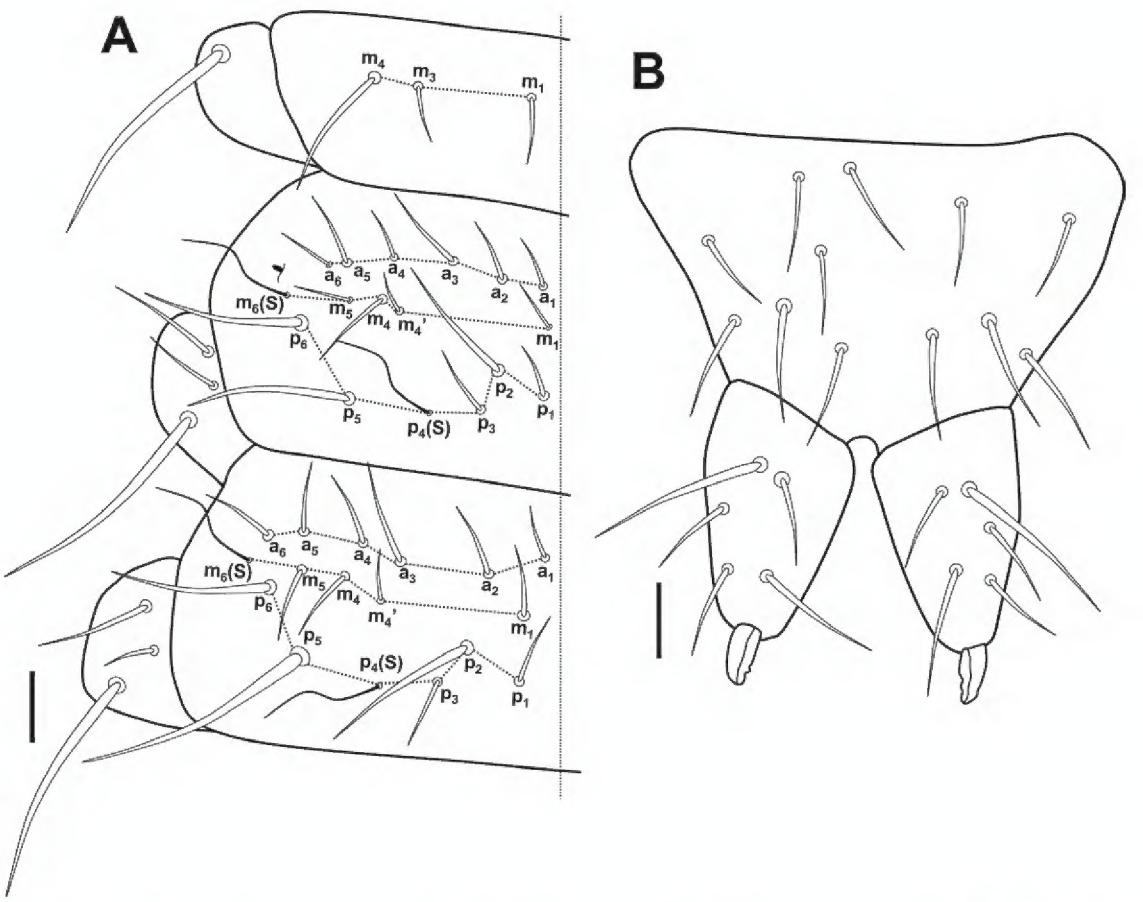

Figure 5. *Schaefferia greensladeae* sp. nov. **A** dorsal side of thorax, setal pattern **B** furca, setal pattern. Scale bars: 0.05 mm (**A**); 0.01 mm (**B**).

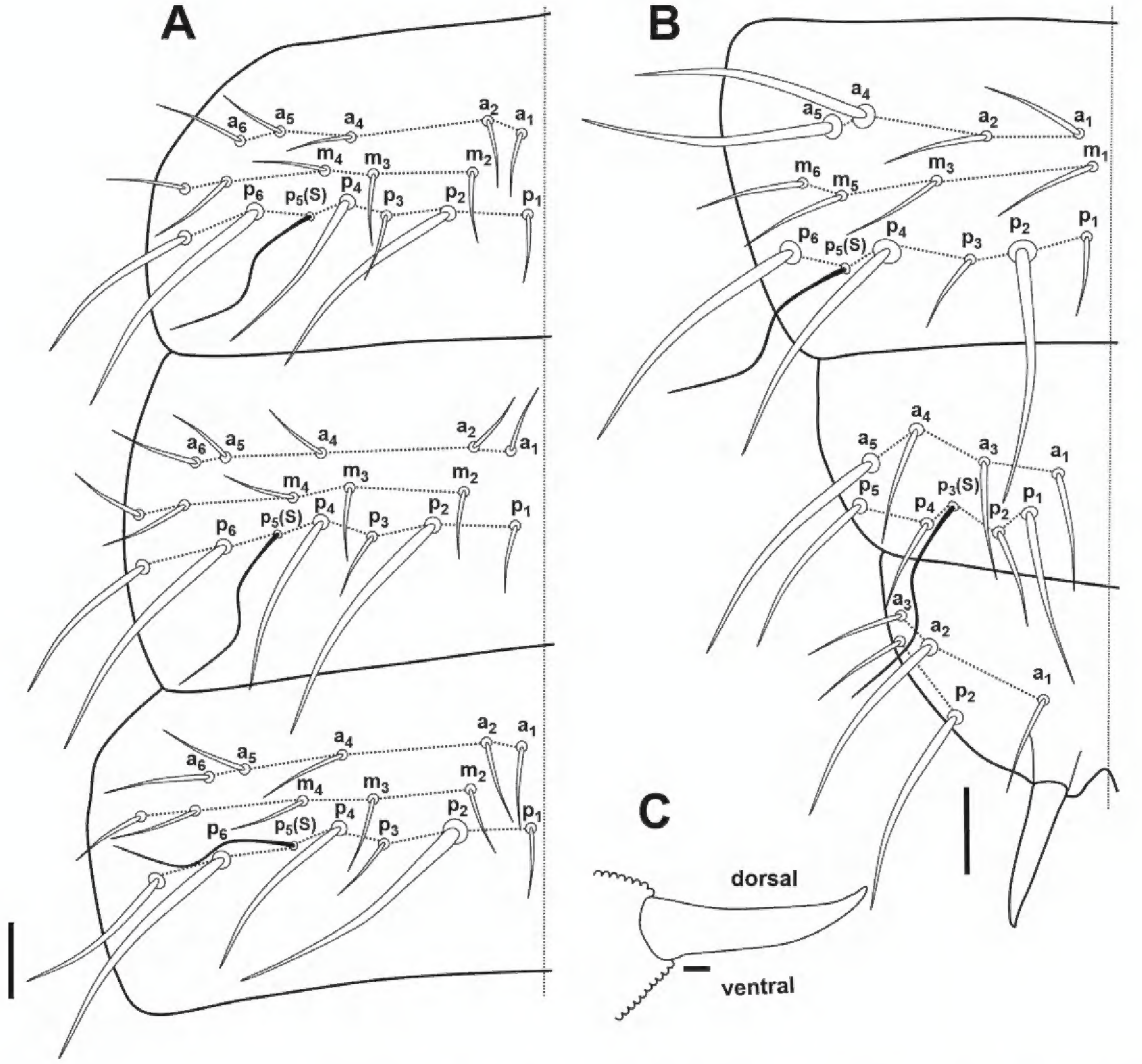

Figure 6. *Schaefferia greensladeae* sp. nov. **A** Dorsal side of Abd I–III, setal pattern **B** dorsal side of Abd IV–VI, setal pattern **C** detail of anal spine, lateral view. Scale bars: 0.05 mm (**A, B**); 0.01 mm (**C**).

basal teeth; pretarsus with 1 lobe and 2 setae (1 external and 1 internal) reaching base of lateral teeth (Fig. 4D–F). Ratio of claw I length: width = 3.2.

Setal pattern of tergites. Dorsal setal pattern generally of type II with well-differentiated setae. Setae on head and body slightly dentated (Fig. 3G). ***Head.*** Setal pattern as in Fig. 3E. Seta a0 present; d1–d5 (d2 and d4 as Mc); sd1–sd5; v1–v2 (v2 as Mc); c1–c5; p1–p3, p5 (p3 and p5 as Mc, p4 absent); oc1–oc3 (oc1 as Mc). ***Thorax.*** Setal pattern as in Fig. 5A. Th I with 3+3 setae as m1, m3 and m4. Th II–III with a1–a6 (a3 as Mc); m1, m4'–m6 (m6 as s), lateral microsensillum present on Th II in front of m6 (s); p1–p4 (p4 as s), p5–p6 (p2, p5 and p6 as Mc). ***Abdomen.*** Setal pattern as in Fig. 6A, B. Abd I–III with 6+6 setae between p5 (s) in rows '*a*' and '*m*': a1, a2, a4, m2, m3 and m4 (p2, p4, p6 and p7 as Mc). Abd IV with 7+7 setae between p5 (s) in rows '*a*' and 'm': a1, a2, a4, a5, m1, m3 and m5 (a4, a5, p2, p4 and p6 as Mc). Abd V with 2+2 setae between p3 (s) on row '*a*': a1 and a3 (a5, p1 and p5 as Mc).

Etymology. The species is named in the honour of prof. Dr. Penelope Greenslade, an outstanding Collembola taxonomist and ecologist.

Taxonomic remarks. Based on absence of eyes, *S. greensladeae* sp. nov. is similar to five representatives of the genus: *S. coeca* Cassagnau, 1959, *S. guerrerense* (Bonet, 1945), *S. oaxacana* Palacios-Vargas & Thibaud, 1985, *S. profundissima* Jordana & Baquero, 2012 and *S. subcoeca* Deharveng & Thibaud, 1980. The new species is characterized by 7 lobes in PAO, while there are 4 (6) lobes in PAO in *S. coeca* and *S. subcoeca*, 4–5 lobes in *S. oaxacana* and 4 lobes in *S. guerrerense* and *S. profundissima*. Moreover, the new species is separated from *S. coeca* in number of sensilla on Ant IV: 8–12 in *S. coeca* and 7–8 in *S. greensladeae*. The both species differ also in number of dental setae: 3–5 setae in *S. coeca* and 5 setae in *S. greensladeae*. Mucro is absent in *S. guerrerense* and *S. subcoeca*, but it is present in the new species. *Schaefferia greensladeae* sp. nov. has 5 dental setae, while *S. oaxacana* has 4 dental setae. Also, *S. greensladeae* sp. nov. in terms of the setal pattern of the dorsal body is close to *S. oaxacana* and *S. profundissima* (see Table 1). *Schaefferia greensladeae* sp. nov. differs from *S. profundissima* by the presence of seta a1 on Abd IV, and from *S. oaxacana* in having seta m4 on Abd IV, whereas *S. oaxacana* has m3. The new species also represents the first record of this genus in the country (Mehrafrooz Mayvan et al. 2023).

Schaefferia montenegrina Kováč & Mehrafrooz Mayvan, sp. nov.

https://zoobank.org/13F15544-55EE-4D2A-AF61-80B6939D2D10

Figs 9–12, Table 1

Diagnosis. Eyes absent, PAO small, forming a rosette of 5–6 regular oval vesicles. Ant IV with 7–8 sensilla and simple apical vesicle. Th I with 2+2 setae, Abd V without seta p2. Claw without internal or lateral teeth. Retinaculum with 3–4 teeth, furca well-developed, with 5 dental setae.

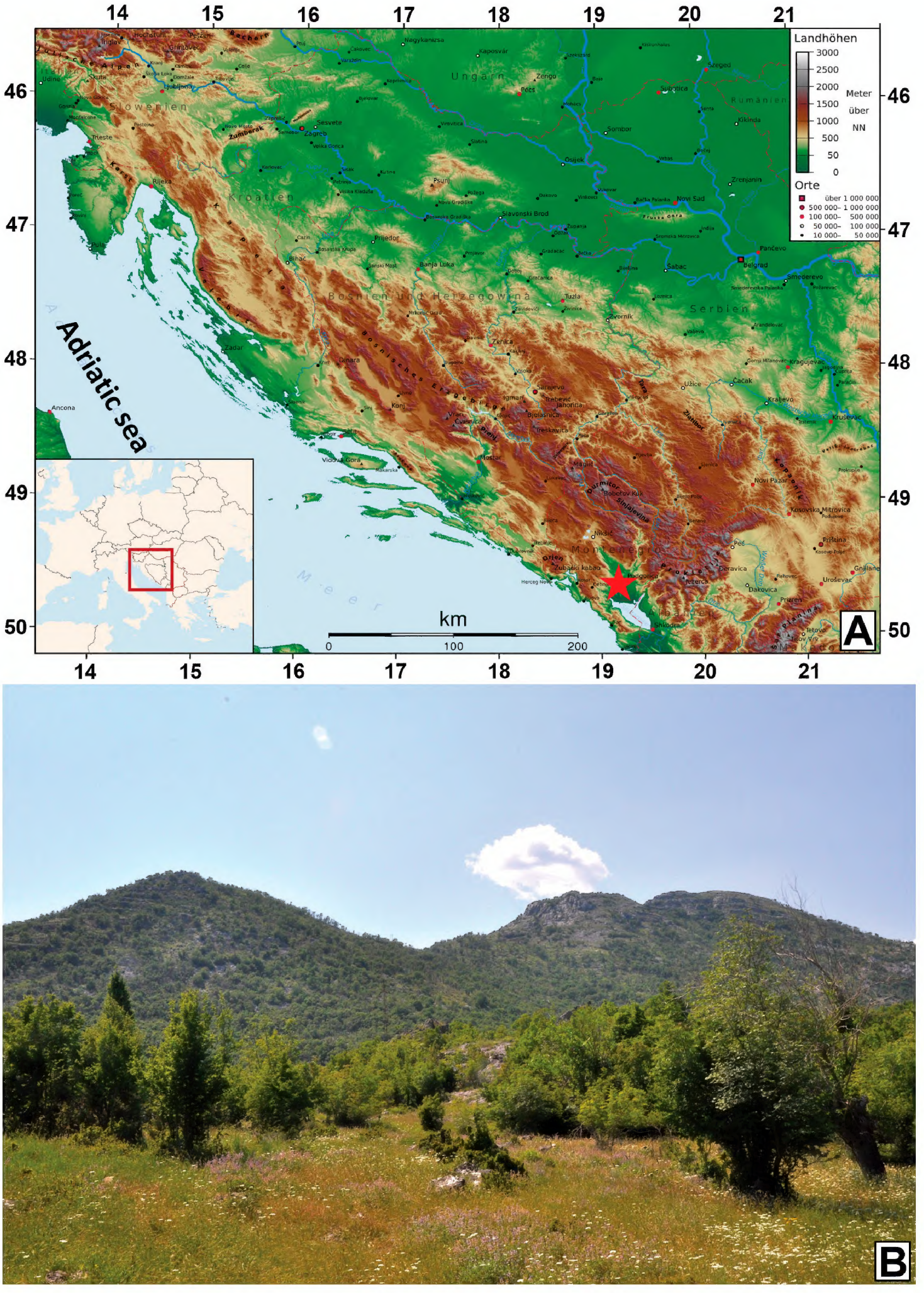

Figure 7. A map of the Dinaric Karst (adapted from www.wikipedia.org), red asterisk: location of the Babatuša Cave in Montenegro – *locus typicus* of *Schaefferia montenegrina* sp. nov. **B** surroundings of the Babatuša Cave (Photo: Ľ. Kováč).

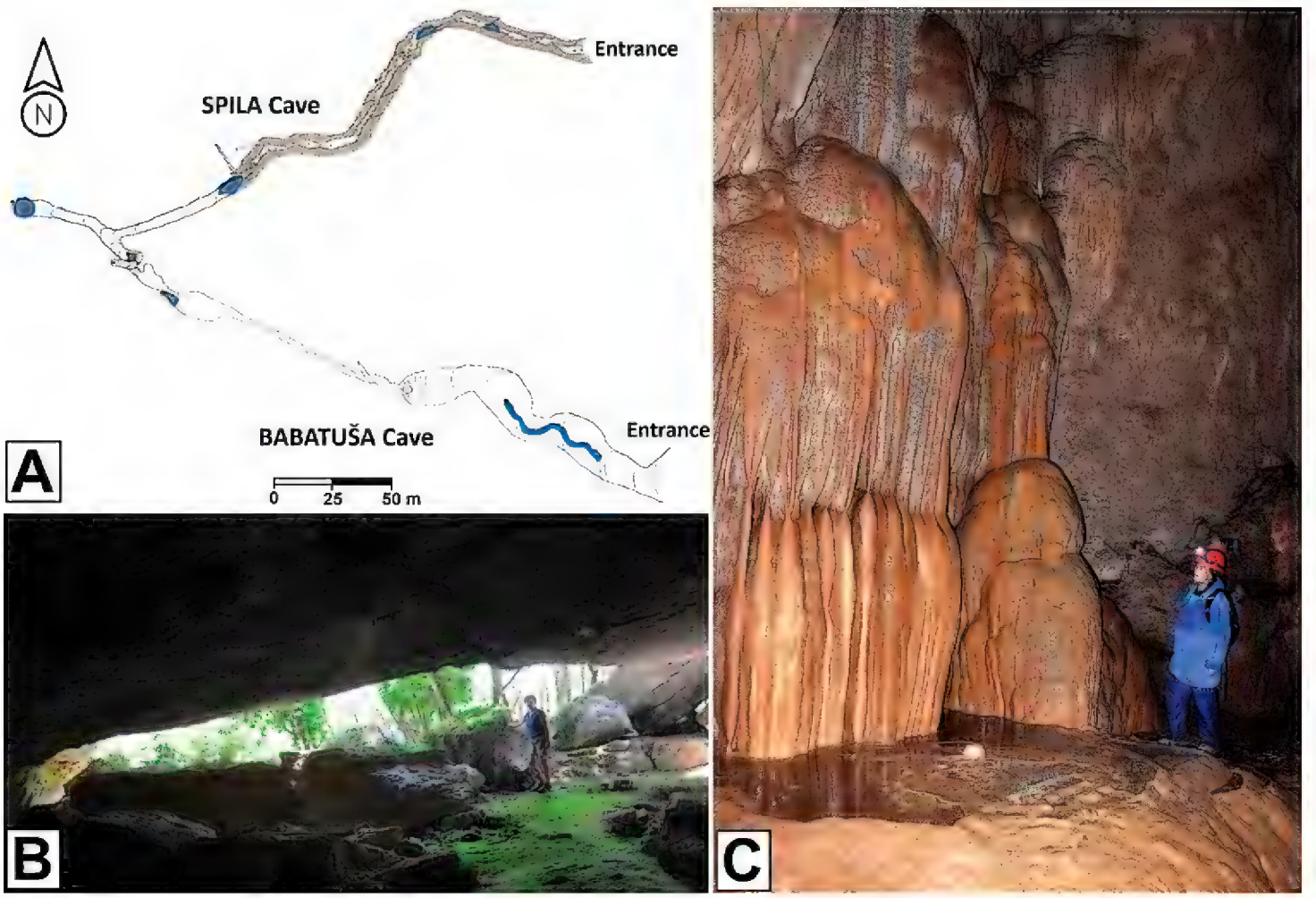

Figure 8. Babatuša Cave, Montenegro **A** ground plan of the cave (adopted from Vujačić et al. 2024) **B** cave entrance (Photo: Ľ. Kováč) **C** flowstones in the main cave chamber (Photo: Ľ. Kováč).

Type locality. Montenegro, Virpazar, Trnovo village, Babatuša Cave, Coordinates: 42°17'56"N, 19°01'58"E, cave entrance 357 m a.s.l.

Type material. ***Holotype.*** • Female on slide [BaSc104MO], dark zone, collected from surface of water, 06.vi.2015, leg. Ľ. Kováč. ***Paratypes.*** • 1 female on slide [BaCa105MO], dark zone, collected from rotten wood, 23.vi.2011, • 1 female on slide [BaCa106MO], dark zone, collected from surface of water, 06.vi.2015, leg. Ľ. Kováč, and • 1 male on slide [BaCa107MO], dark zone, collected from water surface, sinter pool, 23.vi.2011, leg. Ľ. Kováč. Holotype and one paratype deposited in Natural History Museum of Montenegro in Podgorica, and two paratypes deposited in CoPJSU.

Description. ***Body appearance.*** Length 1.4 mm on average (n = 4, max. length 1.5 mm). Habitus typical of *Ceratophysella*, body fusiform. Colour live white without traces of pigment, cuticular granulation rather uniform throughout the body, with well-differentiated setae.

Antenna. It is nearly as long as head, with apical bulb (Fig. 9A–C). Length of Ant I, II, III, IV in holotype as 0.05, 0.04, 0.04, 0.07 mm, respectively. Ant I with 7 setae; Ant II with 7 dorsal and 6 ventral setae in a single row; Ant III and IV clearly separated, Ant III sensory organ with 2 central short and curved rods flanked by 2 guard sensilla (Fig. 9B), small ventrolateral microsensillum in a pit, 10 dorsal and 9 ventral setae;

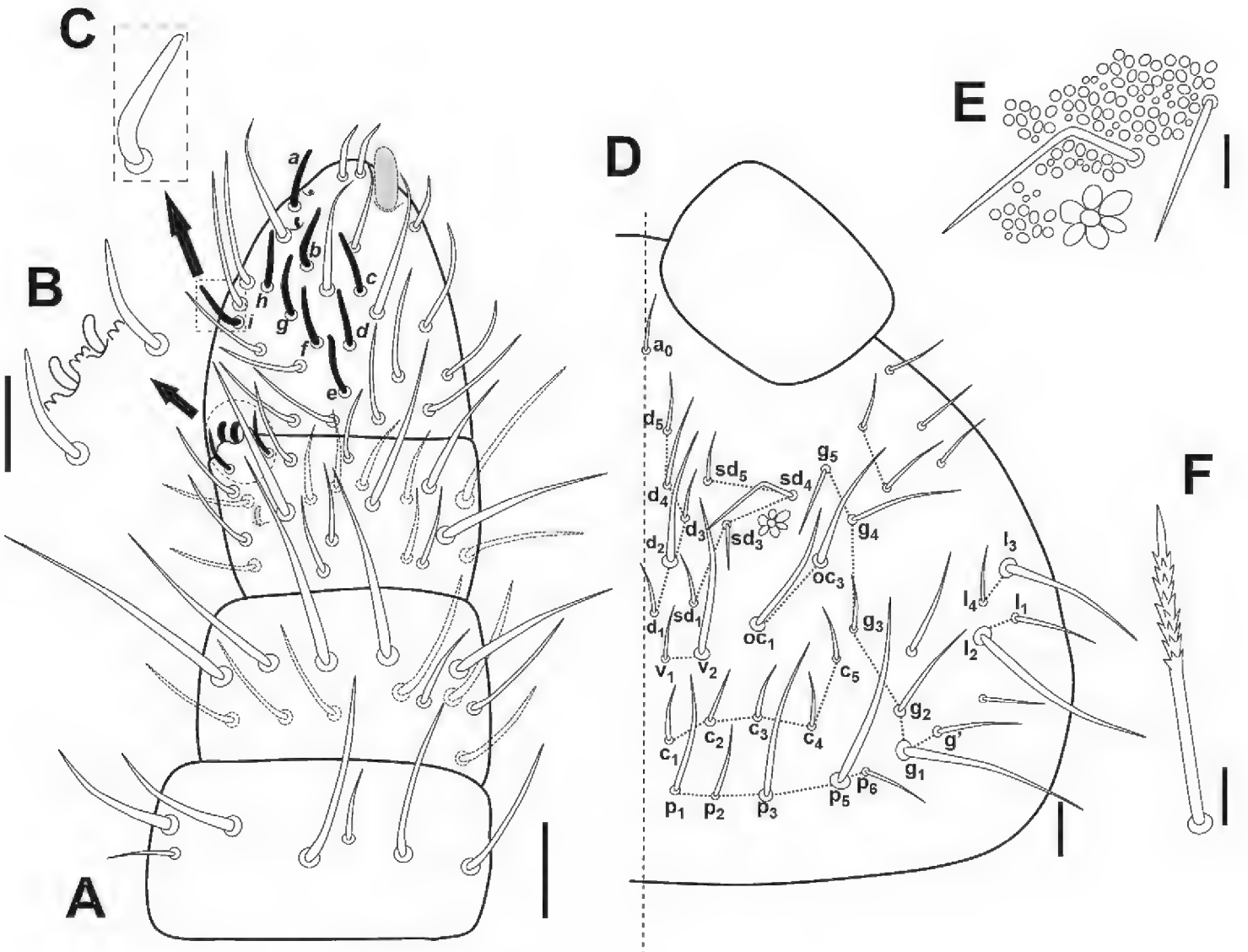

Figure 9. *Schaefferia montenegrina* sp. nov. **A** antennal segments I–IV **B** Ant III sensory organ **C** blunt sensillum on Ant IV **D** dorsal side of head, setal pattern **E** postantennal organ with nearest setae and cuticular granulation **F** shape of macrosetae. Scale bars: 0.025 mm (**A**); 0.01 mm (**B, C**); 0.025 mm (**D**); 0.02 mm (**E–F**).

Ant IV with a simple apical bulb, 7–8 cylindrical blunt sensilla (*a*, *b*, *c*, *d*, *e*, *f*, *g* and *h*), subapical ms near base of sensillum *a*, apical organite os placed between sensilla *a* and *b*, and 18 setae around sensilla.

Head. Eyes absent. PAO small but very distinct and located near base of Ant I, about 17 μm in diameter, with 5–6 equal lobes forming a rosette of 5–6 regular oval vesicles (Fig. 9E). Labrum typical of the genus with 2+2 prelabral setae and 5, 5, 4 labral setae. Labium with all normal papillae (A–E) and 6 proximal setae. Left mandible with 4 teeth and right mandible with 5 teeth.

Abdomen. Ventral tube with 4+4 apical setae. Retinaculum with 3–4 teeth (asymmetry). Furca well-developed as in Fig. 11B, length of manubrium, dens, mucro in holotype as 0.04, 0.04, 0.01 mm respectively; manubrium with 8 setae; dens with 5 setae; mucro about 1/3 of dens length, with lateral lamella, mucro + dens slightly longer than inner edge of claw III. Anal spines shorter than claws (45 μm), on papillae (Fig. 12C). Ratio anal spines: claw III = 0.9; ratio anal spines: dens = 1.

Legs. Tibiotarsi I–III with acuminate tenent hair. Setal pattern as in Fig. 10A–C. Leg I–III with 3, 8, 8 setae on coxae, 7, 7, 7 setae on trochanters, 15, 15, 13 setae on

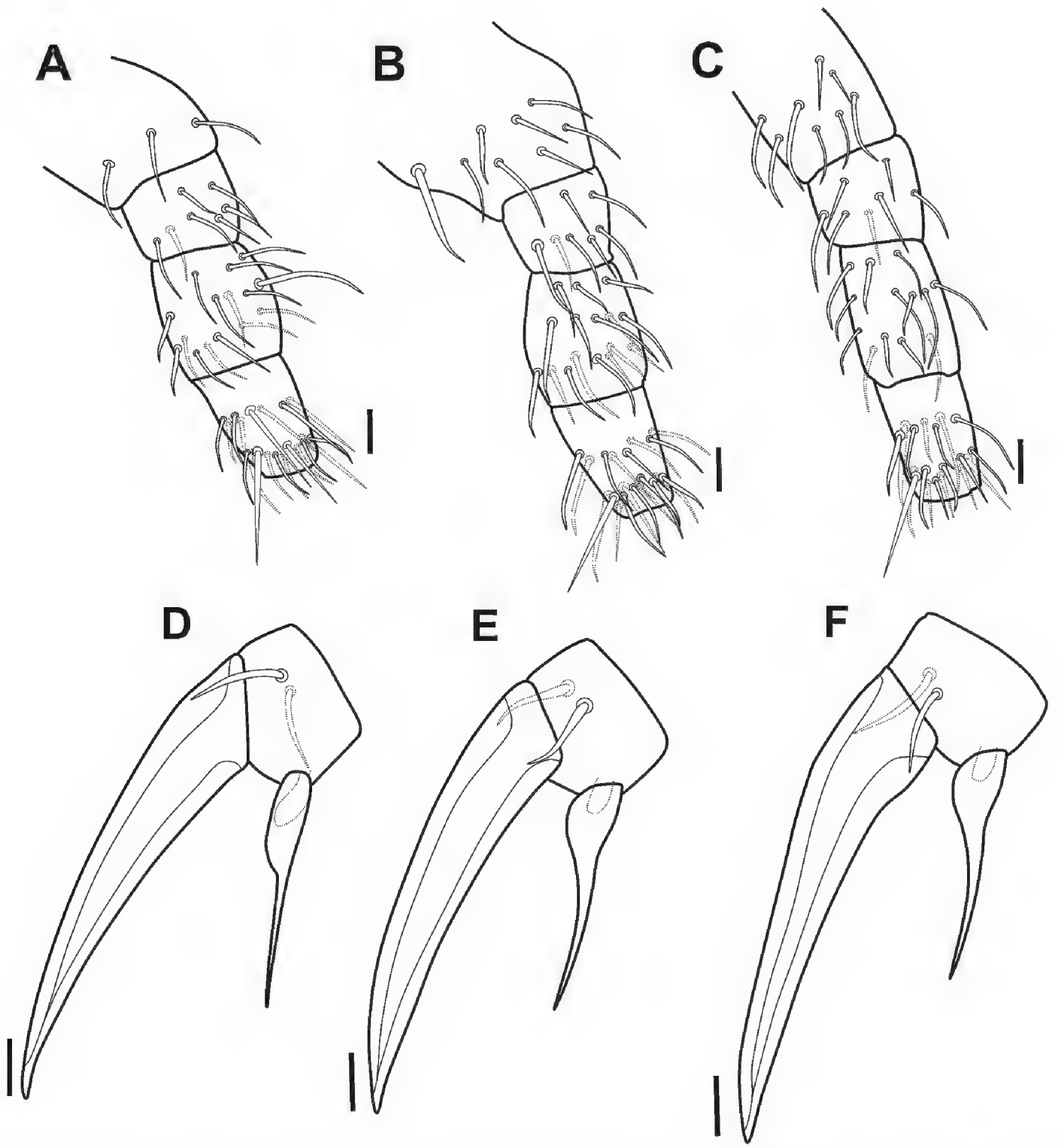

Figure 10. *Schaefferia montenegrina* sp. nov. **A–C** setal pattern of legs **A** leg I **B** leg II **C** leg III **D–F** foot complex of legs **D** leg I **E** leg II **F** leg III. Scale bars: 0.025 mm (**A–C**); 0.01 mm (**D–F**).

femora and 19, 19, 18 setae on tibiotarsi, respectively. Claw slender, 70 μm long in holotype, without internal or lateral teeth; pretarsus with 1 lobe and 2 setae (1 external and 1 internal) (Fig. 10D–F). Ratio of claw I length: width = 5.8.

Setal pattern of tergites. Dorsal setal pattern generally of type II with well-differentiated setae. Setae on head and body finely dentated (Fig. 9F). ***Head.*** Setal pattern as in Fig. 9D. Seta a0 present; d1–d5 (d2 and d4 as Mc); sd1, sd3–sd5; v1–v2 (v2 as Mc); c1–c5; p1–p3, p5 (p3 and p5 as Mc, p4 absent); oc1 and oc3 (oc1 and oc3 as Mc). ***Thorax.*** Setal pattern as in Fig. 11A. Th I with 2+2 setae as m1 and m4 (m3 absent). Th II–III with a1–a6; m1, m4 and m6 (m4 as Mc; m6 as s), lateral microsensillum present on Th II in front of m6 (s); p1–p4 (p4 as s), p5–p6 (p2, p5 and p6 as Mc). ***Abdomen.*** Setal

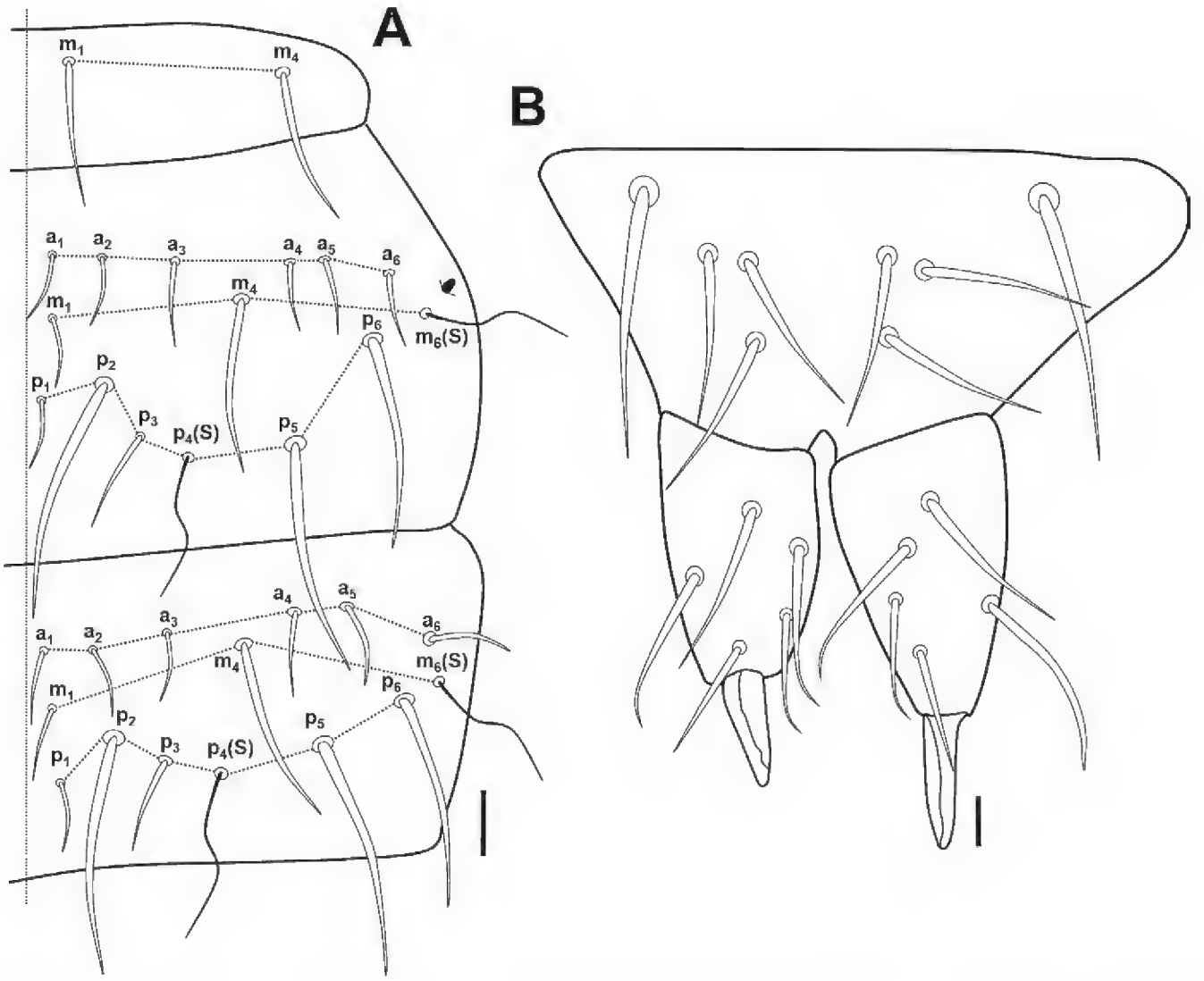

Figure 11. *Schaefferia montenegrina* sp. nov. **A** dorsal side of thorax **B** furca, setal pattern. Scale bars: 0.05 mm (**A**); 0.01 mm (**B**).

pattern as in Fig. 12A, B. Abd I with 4+4 setae between p5 (s) in rows '*a*' and '*m*': a1, a2, a5 and m3 (p2, p4, p6 and p7 as Mc); Abd II with 5+5 setae between p5 (s) in rows '*a*' and '*m*': a1, a2, a4, a5 and m3 (p2, p4, p6 and p7 as Mc); Abd III with 6+6 setae between p5 (s) in rows '*a*' and '*m*': a1, a2, a4, m3 and m4 (p2, p4, p6 and p7 as Mc). Abd IV with 5+5 setae between p5 (s) in rows '*a*' and '*m*': a1, a2, a4, and m1 (a5, p2, p4 and p6 as Mc). Abd V with 2+2 setae between p3 (s) on row '*a*': a1 and a3 (a5, p1 and p5 as Mc).

Etymology. The name of the new species is derived from the Montenegro country in Europe, where it was discovered.

Taxonomic remarks. Similarly to *S. montenegrina* sp. nov. the following species have reduced number of eyes to 0–1: *S. greensladeae* sp. nov., *S. coeca*, *S. guerrerense*, *S. oaxacana*, *S. profundissima* and *S. subcoeca*. *Schaefferia montenegrina* sp. nov. differs from *S. greensladeae* sp. nov. in size and number of PAO lobes: *S. montenegrina* sp. nov. has a small PAO with 5–6 subequal lobes, while *S. greensladeae* sp. nov. has large PAO with 7 unequal lobes. Moreover, *S. montenegrina* sp. nov. is separated from *S. guerrerense* and *S. subcoeca* in having a mucro, while mucro is absent in the latter two species. Other differences such as the presence of 2+2 setae on Th I, absence of seta p2 on abd V, number of sensilla on Ant IV, number of dental setae, presence of an internal tooth on unguis and setal pattern of body tergites, also separate the new species from others (see Table 1).

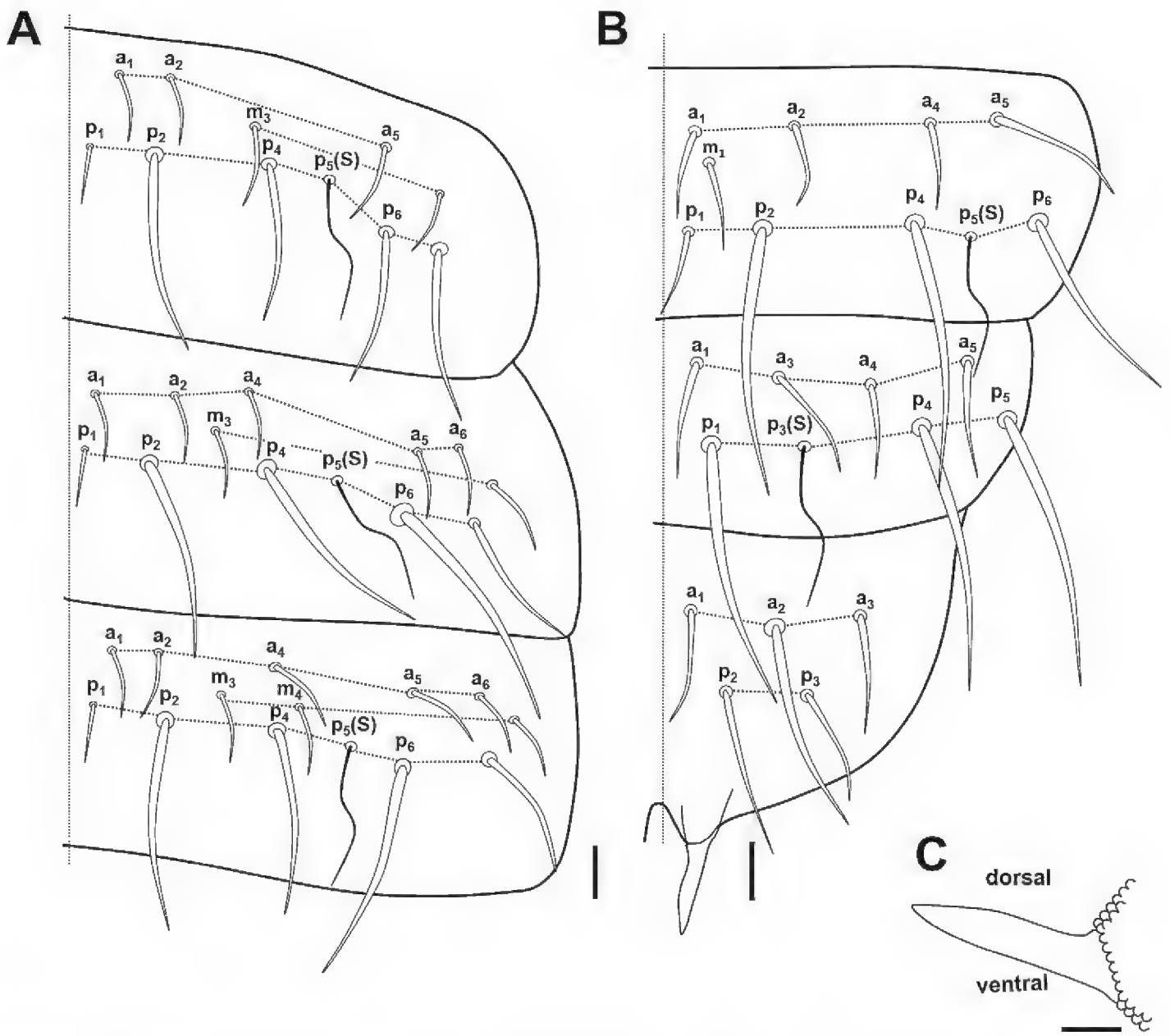

Figure 12. *Schaefferia montenegrina* sp. nov. **A** Dorsal side of Abd I–III, setal pattern **B** dorsal side of Abd IV–VI, setal pattern **C** detail of anal spine, lateral view. Scale bars: 0.05 mm (**A, B**); 0.01 mm (**C**).

Table 1. Differential characters of eyeless *Schaefferia* species. Characters: 1, Number of eyes; 2, PAO lobes number; 3, Ant IV S number; 4, mucro: 0 = absent, 1 = present, 2 = reduced; 5, Dental setae number; 6, Tenaculum teeth number; 7, Claw internal teeth: 0 = absent, 1 = present; 8, Empodial lamella; 9, Abd II–III *a*-row setae; 10, Abd II–III *m*-row setae; 11, Abd IV *a*-row setae; 12, Abd IV *m*-row setae; 13, Abd V *a*-row setae; 14, References used for the data presented: 1, Bonet 1945; 2, Cassagnau 1959; 3, Thibaud 1972; 4, Deharveng and Thibaud 1980; 5, Palacios-Vargas and Thibaud 1985; 6, Thibaud et al. 2004; 7, Jordana et al. 2012. "-"= data absent.

Species/Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Schaefferia coeca Cassagnau, 1959	0–1	4(6)	8–12	2	3–5	3	-	-	1 2 4	2 4	1 3 5	1 4	1 3	2, 3, 6
Schaefferia greensladeae sp. nov.	0	7	8	1	5	3–4	1	yes	1 2 4	2 3 4	1 2 4 5	1 3 5	1 3	Present study
Schaefferia guerrerense (Bonet, 1945)	0	4	6–7	0	4(5)	3	1	yes	1 2 4	2 3 5	1 3 4 5	1 2 4 5	1 3	1, 3
Schaefferia montenegrina sp. nov.	0	5–6	7–8	1	5	4	0	yes	1 2 4 (5)	3 (4)	1 2 4	1	1 3	Present study
Schaefferia oaxacana Palacios-Vargas & Thibaud, 1985	0	4–5	8	1	4	3–4	1	yes	1 2 4 5	2 3 5	1 2 4 5	1 4 5	1 3	5
Schaefferia profundissima Jordana & Baquero, 2012	0–1	4	7	1	5	4	1	-	1 2 4 (5)	2 3 (4)	2 3 4 5	1 4 5	1 3	7
Schaefferia subcoeca Deharveng & Thibaud, 1980	0	4(5)	7	0	3	3	-	-	1 2 4 5	2 4	1 3 5	1 3 5	1 3	4, 6

Remarks to phylogeny of the genus

Thibaud (1972) outlined *Schaefferia* as a valid genus, representatives of which show morphological regressions in pigmentation, number of eyes and furcal complex. He regarded these features as manifestations of adaptation to the eudaphic or cave lifestyle, having evolutionary significance, and the genus as a phyletic line of the species group *Ceratophysella denticulata*, formed as a result of postglacial colonization of the underground environment (deep soil, caves). The genus *Schaefferia*, similarly as genera *Typhlogastrura* Bonet, 1930 and *Bonetogastrura* Thibaud, 1974, is apparently derived from *Ceratophysella* groups of species, which have successfully invaded caves and became more or less troglomorphic (Christiansen and Wang 2006). In the contrary to *Schaefferia*, the both genera are characterized by the maintenance of a fully developed furca. After Christiansen (1985), reduction of furca in *Schaefferia* may represent an edaphic phenomenon. It is important to note that all three genera have common Holarctic distribution.

However, any assumptions to phyletic relationships within the genus *Schaefferia*, or presumably related genera, remain to be clarified by a thorough phylogenetic study combining morphological traits with mitochondrial and nuclear DNA data. It is indeed not an easy task when considering demanding field collections of these collembolans with usually rare and dispersed distribution in subterranean spaces.

Remarks on morphological adaptations to subterranean environment

In the previous section, we briefly discussed troglomorphic and edaphomorphic adaptations in *Schaefferia* representatives. Thus, blidness itself doesn't necessarily imply association with cave life, well documented in this genus by three edaphic species, all from France: *S. ariegica* Cassagnau, 1959, *S. coeca* and *S. subcoeca*. Regarding troglobiotic and guanobiotic forms, altogether six species have been reported in this genus exclusively from caves (Lukić 2019): *S. guerrerense* and *S. oaxacana* from Mexico, *S. profundissima* from the Caucasus, *S. kitakamiana* Yosii, 1991 from Japan, *S. baschkirica* Kniss, 1985 from the Urals, Russia, and *S. hubbardi* Thibaud, 1995 from USA. *Schaefferia montenegrina* sp. nov. has clearly elongated claw and missing internal teeth, the combination of both characters considered troglomorphic adaptations (Christiansen 2012). It has been noticed that some guanobiotic species of the family Hypogastruridae exhibit this character (Deharveng and Bedos 2018) as an adaptation to easier movement on fresh bat guano (Lukić 2019), such as in *Acherontides eleonorae* Palacios-Vargas and Gnaspini-Netto, 1992 from Brazil. Nevertheless, there are three blind species of *Schaefferia* with elongated unguis: two species described in this paper and *S. profundissima* from Krubera-Voronya, a deep cave system in the Caucasus Mountains. Elongation of unguis (claw) in these species is an obvious troglomorphic character that evolved independently in three geographically distant karst complexes (Dinaric Karst, Alborz Mountains and Caucasus Mountains) as an example of convergent evolution, a well-known phenomenon in several invertebrate groups inhabiting deep subterranean realm.

Key to world species of genus *Schaefferia*

The following key was prepared based on the diagnostic characters of worldwide *Schaefferia* species (adapted from Jordana et al. 2020).

1 Mucro absent **2**
– Mucro reduced or well-developed **9**
2 With 7+7 eyes; dens with 6 setae; Abd IV without seta a1 ..... ***S. oculea* Babenko, 1999 (Russia, soil)**
– With less than 7+7 eyes; dens with less than 6 setae; Abd IV with seta a1 ... **3**
3 With 5+5 eyes .... ***S. canigouensis* Deharveng & Thibaud, 1980 (France, soil)**
– With less than 5+5 eyes **4**
4 With 3+3 eyes **5**
– With less than 3+3 eyes **6**
5 Dens with 4 setae; Abd II–III with 6+6 setae in *a*- and *m*-row together (m3 present) ..... ***S. emucronata* Absolon, 1900 (Czech Republic, cave)**
– Dens with 3 setae; Abd II–III with 5+5 setae in *a*- and *m*-row together (m3 absent) ..... ***S. lindbergi* Gama, 1963 (Portugal, cave)**
6 With 2+2 eyes **7**
– With 0+0 eyes **8**
7 Dens with 4 setae; Ant IV with 8–9 sensilla; Abd II–III without setae in *m*-row; Abd IV without seta a4 ..... ***S. quadrioculata* (Stach, 1939) (Germany, cave)**
– Dens with 3 setae; Ant IV with 7 sensilla; Abd II–III with 2 setae in *m*-row (m2 and m4); Abd IV with seta a4 ..... ***S. ariegica* Cassagnau, 1959 (France, soil)**
8 Dens with 4–5 setae; Abd IV with seta a4; Abd IV with setae a1, a2, a4, a5 . ..... ***S. guerrerense* (Bonet, 1945) (Mexico, cave)**
– Dens with 3 setae; Abd IV without seta a4; Abd IV with setae a1, a3, a5 ..... ***S. subcoeca* Deharveng & Thibaud, 1980 (France, soil)**
9 Mucro reduced **10**
– Mucro well-developed **15**
10 With 5+5 eyes **11**
– With less than 5+5 eyes **12**
11 Dens with 5 setae; Abd II–III with 2+2 setae in *m*-row (m2, m4) ..... ***S. maxima* Deharveng & Thibaud, 1980 (France, soil)**
– Dens with 3–4 setae; Abd II–III with 3+3 setae in *m*-row (m2, m3, m4) ..... ***S. decemoculata* (Stach, 1939) (Germany, cave)**
12 With 4+4 eyes ..... ***S. willemi* (Bonet, 1930) (Belgium, cave)**
– With less than 4+4 eyes **13**
13 With 3+3 eyes **14**
– With 0–1 eye ..... ***S. coeca* Cassagnau, 1959 (France, soil)**

14 Dens with 3 setae; PAO with 4 vesicles; Abd II–III without setae in *m*-row; Abd V without seta a2 ..... ***S. pouadensis* Delamare Deboutteville, 1945 (France, cave)**

– Dens with 6 setae (sometime 5); PAO with 6–8 vesicles; Abd II–III with 2+2 setae in *m*-row (m2, m4); Abd V with seta a2 ..... ***S. scossirolii* Dallai & Sabatini, 1981 (Italy, soil)**

15 With 6+6 eyes..... **16**

– With less than 6+6 eyes..... **24**

16 Ant IV with 7 sensilla **17**

– Ant IV with 6 sensilla **23**

17 Dens with 3–4 setae..... ***S. cheoha* Wray, 1963 (USA, cave)**

– Dens with more than 4 setae..... **18**

18 Abd V with seta a2..... ***S. fjellbergi* Jordana & Baquero, 2020 (USA, soil)**

– Abd V without seta a2 ..... **19**

19 Abd IV with 5+5 setae in *a*-row (a1–a5) ..... ***S. vandalica* (Jordana & Arbea, 1990) (Spain, soil)**

– Abd IV with less than 5+5 setae in *a*-row..... **20**

20 Abd IV with 3+3 setae in *m*-row **21**

– Abd IV with 2+2 setae in *m*-row **22**

21 Abd II–III with setae m2, m4, m5; dens with 5 setae; ratio Asp: dens as 4.5; ratio dens: mucro as 4..... ***S. babenkoi* Jordana & Baquero, 2020 (Russia, soil)**

– Abd II–III with setae m2, m3, m4; dens with 5–6 setae; ratio Asp: dens as 2.3; ratio dens: mucro as 2.7 ..... ***S. duodecimocellata* Bonet, 1945 (Mexico, soil)**

22 Dens with 5 setae; Abd IV with setae a1, a3, a5 in *a*-row; Abd IV with setae m1, m4 in *m*-row; ratio Asp: dens as 1; ratio dens: mucro as 2.3 ..... ***S. elegans* (Cassagnau, 1959) (France, soil)**

– Dens with 6 setae; Abd IV with setae a1, a2, a4, a5 in *a*-row; Abd IV with setae m1, m5 in *m*-row; ratio Asp: dens as 2.2; ratio dens: mucro as 2.5 ..... ***S. sendrai* Jordana & Baquero, 2020 (Spain, soil)**

23 Dens with 4 setae; tenaculum with 3+3 teeth; Abd IV with 2+2 setae in *m*-row (m1, m3) ..... ***S. deharvergi* (Jordana & Arbea, 1990) (Spain, soil)**

– Dens with 5 setae; tenaculum with 4+4 teeth; Abd IV with 1+1 seta in *m*-row (m1) ..... ***S. jarae* (Jordana & Arbea, 1990) (Spain, soil)**

24 With 5+5 eyes..... **25**

– With less than 5+5 eyes..... **29**

25 Ant IV with 5 sensilla; dens with 3 setae ..... ***S. bidentata* (Cassagnau, 1954) (France, soil)**

– Ant IV with more than 5 sensilla; dens with more than 3 setae..... **26**

26 PAO with 5 vesicles; Abd V without seta p1 .....
***S. raulmugnizi* Palacios-Vargas & Castaño-Meneses, 2008 (Mexico, soil)**
– PAO with 4 vesicles; Abd V with setae p1 ..... **27**
27 Dens with 4–5 setae; Abd IV without seta a1 .....
..... ***S. bermani* Babenko, 2019 (Russia, soil)**
– Dens with 5–6 setae; Abd IV with seta a1 ..... **28**
28 Labial papilla C absent; Abd V without seta a2' .....
..... ***S. czernovi* (Martynova, 1978) (Russia, soil)**
– Labial papilla C present; Abd V with seta a2' .....
..... ***S. quinqueoculata* (Yosii, 1956) (Japan, cave)**
29 With 4+4 eyes **30**
– With less than 4+4 eyes **31**
30 PAO with 4 vesicles; Ant IV with 6–7 sensilla; Abd IV with 4+4 setae in *m*-row (m1, m3, m4, m5) ..... ***S. fukugakuchiana* (Yosii, 1956) (Japan, cave)**
– PAO with 4–6 (7–8) vesicles; Ant IV with 7–8 sensilla; Abd IV with 1+1 seta in *m*-row (m4) ..... ***S. baschkirica* Kniss, 1985 (Russia, cave)**
31 With 3+3 eyes **32**
– With less than 3+3 eyes **33**
32 Ant IV with 6–7 sensilla; dens with 5 setae; Abd II–III with 3+3 setae in *m*-row (m2, m3, m5) ..... ***S. hubbardi* Thibaud, 1995 (USA, cave)**
– Ant IV with 9 (7–8) sensilla; dens with 4 setae; Abd II–III without setae in *m*-row ..... ***S. sexoculata* (Gisin, 1947) (Switzerland, soil)**
33 With 2+2 eyes; Ant IV with 6 sensilla .....
..... ***S. kitakamiana* Yosii, 1991 (Japan, cave)**
– With less than 2+2 eyes; Ant IV with more than 6 sensilla 34
34 Ant IV with trilobed apical vesicle; dens with 4 setae; Ti I–III with 18, 18, 17 setae, respectively .....
..... ***S. oaxacana* Palacios-Vargas & Thibaud, 1985 (Mexico, cave)**
– Ant IV with simple apical vesicle; dens with 5 setae; Ti I–III with 19, 19, 18 setae, respectively **35**
35 PAO with 4 vesicles; dorsal head without seta a0; Abd IV without seta a1 in *a*-row ..... ***S. profundissima* Jordana & Baquero, 2012 (Georgia, cave)**
– PAO with more than 4 vesicles; dorsal head with seta a0; Abd IV with seta a1 in *a*-row **36**
36 PAO with 7 vesicles; Th I with 3+3 setae (m1, m3, m4); Abd V with seta p2; Cl with a strong inner tooth and pair of lateral teeth .....
..... ***S. greensladeae* sp. nov. (Iran, cave)**
– PAO with 5–6 vesicles; Th I with 2+2 setae (m1, m4); Abd V without seta p2; Cl without inner or lateral teeth ..... ***S. montenegrina* sp. nov. (Montenegro, cave)**

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References

Absolon K (1900) Vorläufige Mittheilung über einige neue Collembolen aus den Höhlen. Zoologischer Anzeiger 23: 265–269.

Babenko AB (1999) New springtail species of the family Hypogastruridae from Eastern Siberia. Zoologichesky Zhurnal 78: 885–888.

Babenko AB (2019) A new species of *Schaefferia* Absolon (Collembola: Hypogastruridae) from Chukotka, Russia, with remarks on the related forms. Zootaxa 4571(2): 247–254. https://doi.org/10.11646/zootaxa.4571.2.4

Barjadze S, Mehrafrooz Mayvan M, Parimuchová A, Maghradze E, Kováč Ľ (2024) Two new species of *Plutomurus* Yosii (Collembola: Tomoceridae) from Georgia and Iran. Zootaxa 5463(4): 559–572. https://doi.org/10.11646/zootaxa.5463.4.6

Bonet F (1930) Remarques sur les Hypogastruriens cavernicoles avec descriptions d'espèces nouvelles (Collembola). EOS, Revista Española de Entomología 6(2): 113–139.

Bonet F (1945) Nuevos Generos y Especies de Hipogastruridos de Mexico (Collembola). Revista de la Sociedad Mexicana de Historia Natural 6(2): 13–45.

Börner C (1932) Apterygota. In: Brohmer P (Ed.) Fauna Von Deutschland. 4th edn. Queller & Meyer, Leipzig, 136–143.

Cassagnau P (1954) Faune française des Collemboles. III. Poduromorphes de haute montagne. Vie et Milieu 4(1): 65–74.

Cassagnau P (1959) Faune française des Collemboles IX. Les *Hypogastrura* a. l. du Massif du Néouvielle (Hautes-Pyrénées). Remarques sur chetotaxie des espèces. Vie et Milieu 9(4): 476–503.

Christiansen KA (1985) Regressive evolution in Collembola. National Speleological Society Bulletin 47(2): 89–100.

Christiansen KA (2012) Morphological adaptations. Pp. 517–528. In: White WB, Culver DC (Eds) Encyclopedia of caves. Elsevier/Academic Press, 945 pp. https://doi.org/10.1016/B978-0-12-383832-2.00075-X

Christiansen KA, Wang H (2006) A revision of the genus *Typhlogastrura* in North American caves with description of five new species. Journal of Cave and Karst Studies 68(2): 85–98.

Dallai R, Sabatini MA (1981) Ricerche Sui Collemboli XXVIII. Su di una nuova specie endogea di *Schaefferia*. Redia 64: 195–204.

Deharveng L, Bedos A (2018) Chapter 7. Diversity of terrestrial invertebrates in subterranean habitats. In: Moldovan OT, Kováč Ľ, Halse S (Eds) Cave Ecology. Ecological Studies Vol. 235. Springer, Cham, 107–172. https://doi.org/10.1007/978-3-319-98852-8_7

Deharveng L, Thibaud JM (1980) Trois espèces nouvelles du genre *Schaefferia* Absolon, 1900 (Insecte, Collembole). Nouvelle Revue d'Entomologie 10: 3–10.

Delamare-Deboutteville C (1945) Sur quelques Collemboles de la région de Banyuls (Pyrénées-Orientales) avec la description d'une espèce troglobie. Bulletin de la société entomologique de France 6: 70–72. https://doi.org/10.3406/bsef.1945.15827

Djurovic M, Djurovic P (2021) Review of the most significant caves in Montenegro. Acta Carsologica 50(1): 49–65. https://doi.org/10.3986/ac.vi.7182

Fjellberg A (1985) Arctic Collembola. I – Alaskan Collembola of the families Poduridae, Hypogastruridae, Odontellidae, Brachystomellidae and Neanuridae. Entomologica Scandinavica 21: 1–126.

Gama MM da (1963) Quatre espèces nouvelles de Collemboles d'Autriche et de Jougoslavie. Archives des Sciences (Genève) 16: 43–50.

Ghelich Khani P, Qashqaei AT, Kolkert H (2024) Status of vertebrate species in Danial Cave, northern Iran. Oryx 58(1): 104–109. https://doi.org/10.1017/S003060532200165X

Gisin H (1947) Sur les insectes aptérygotes du Parc National Suisse. Espèces et groupements euédaphiques. Résultats des recheerches scientifiques enterprises au Parc Natational Suisse 2: 77–91.

Jordana R, Arbea JI (1990) Especies ibéricas de *Hypogastrura* (*Ceratophysella*) de seis ojos con descripción de tres nuevas especies (Collembola, Hypogastruridae). Graellsia 46: 161–173.

Jordana R, Arbea JI, Simon JC, Lucianez MJ (1997) Collembola Poduromorpha. In: Ramos MA, et al. (Eds) En: Fauna Iberica, 8. Museo Nacional de Ciencias Naturales (CSIC), Madrid, 807–807.

Jordana R, Baquero E (2012) Reviews of the genera *Schaefferia* Absolon, 1900, *Deuteraphorura* Absolon, 1901, *Plutomurus* Yosii, 1956 and the *Anurida* Laboulbène, 1865 species group without eyes, with the description of four new species of cave springtails (Collembola) from Krubera-Voronya cave, Arabika Massif, Abkhazia. Terrestrial Arthropod Reviews 5: 35–85. https://doi.org/10.1163/187498312X622430

Jordana R, Baquero E, Ledesma E, Sendra A, Ortuño VM (2020) Poduromorpha (Collembola) from a sampling in the mesovoid shallow substratum of the Sierra de Guadarrama National Park (Madrid and Segovia, Spain): Taxonomy and Biogeography. Zoologischer Anzeiger 285: 81–96. https://doi.org/10.1016/j.jcz.2020.02.001

Kniss VA (1985) Springtails of the genus *Schaefferia* (Collembola, Hypogastruridae) from caves in the southwestern part of the Bashkir ASSR. Zoologicheskii zhurnal 64(6): 934–937.

Lukić M (2019) Chapter 35 - Collembola. 308–319. In: White WB, Culver D, Pipan T (Eds) Encyclopedia of Caves, 3rd edn., Academic Press, 1250 pp. https://doi.org/10.1016/B978-0-12-814124-3.00034-0

Martynova EF (1978) New species of Springtails of the genus *Hypogastrura* s. l. in the North-Eastern Asia Fauna. Novye I Maloizvestnye Vidy Fauny Sibiri 12: 27–47.

Mehrafrooz Mayvan M, Greenslade P, Sadeghi-Namaghi H (2023) An annotated checklist of the Collembola (Hexapoda) from Iran. Zootaxa 5275(1): 001–101. https://doi.org/10.11646/zootaxa.5275.1.1

Mehrafrooz Mayvan M, Kováč Ľ (2025) Two new species of the genus *Acherontiella* (Collembola, Hypogastruridae) from caves of the Alborz and Kopet Dagh mountains in Iran. ZooKeys 1255: 1–25. https://doi.org/10.3897/zookeys.1255.164170

Palacios-Vargas JG, Castaño-Meneses G (2008) A new species of *Schaefferia* Absolon, 1900 (Collembola: Hypogastruridae). Dugesiana 15(2): 123–125.

Palacios-Vargas JG, Gnaspini-Netto P (1992) A new Brazilian species of *Acherontides* (Collembola: Hypogastruridae) with notes on its ecology. Journal of The Kansas Entomological Society 65: 443–447.

Palacios-Vargas JG, Thibaud JM (1985) Nuevos Hypogastruridae anoftalmos (Collembola) de cuevas y suelos de México. Folia Entomologica Mexicana 66: 3–16.

Stach J (1939) Die Höhlenfauna des Glatzer Schneeberges. II Die Collembolenfauna der Salzlöcher bei Seitendorf. Beiträge zur Biologie des Glatzer Schneeberges 5: 395–415.

Steiner W (1955) Beiträge zur Kenntnis der Collembolenfauna Spaniens. Eos 34: 323–340.

Thibaud JM (1972) Revision du genre *Schaefferia* Absolon, 1900 (Insecte, Collembole). Annales de Spéléologie 27(2): 363–394.

Thibaud JM (1974) Subdivision de *Typhlogastrura*, genre troglobie de la famille des Hypogastruridae (Insectes Collemboles). Spelunca 8: 205–207.

Thibaud JM (1995) Une nouvelle espèce de *Schaefferia* d'une grotte des Etats-Unis d'Amérique (Collembola, Hypogastruridae). Revue Française d'Entomologie (Nouvelle Série) 17(2): 77–78.

Thibaud JM, Schulz HJ, Gama Assalino MM da (2004) Hypogastruridae. In: Dunger W (Ed.) Synopses on Palaearctic Collembola. Vol. 4, Abhandlungen und Berichte des Naturkundemuseums Görlitz 75(2): 1–287.

Vujačić D, Perović Đ, Grozdanić G, Vujović F (2024) Protected Speleological Objects. In: Barovic G (Eds) Speleology of Montenegro. Cave and Karst Systems of the World. Springer, Cham. https://doi.org/10.1007/978-3-031-49375-1_8

Wray DL (1963) Collembola of North America; Part I, Hypogastrurinae. Journal of the Tennessee Academy of Science 38(3): 101–104.

Yosii R (1991) About the proserpinae group of *Hypogastrura* (Collembola) in the Caves of Pref. Iwate. Annals of the Speleological Research Institute of Japan (Iwaizumi) 9: 3–10.

Yosii R (1956) Monographie zur Höhlencollembolen japans. Contributions from the Biological Laboratory Kyoto University 3: 1–109.

Yosii R (1962) Studies on the collembolan genus *Hypogastrura* II. Nearctic forms collected by Prof. F. Bonet. Contributions from the Biological Laboratory, Kyoto University 13: 1–25.

Yusefinia AA (2017) Ancient Langa: Kelarabad, Salmanshahr and Abas Abad. Bolur Publisher, Rasht, 61 pp. [In Persian]